

EM 199907  
EW 19990705

L10 ANSWER 18 OF 41 MEDLINE

AN 97146199 MEDLINE

DN 97146199

TI Treatment of hypercholesterolemia in patients undergoing multiple coronary angioplasties.

AU Marques V; Bowser S; Hendrickxs J; Ruffner R

CS Dept. of Medicine, Shadyside Hospital, Pittsburgh, Pennsylvania 15232, USA.

SO REVISTA PORTUGUESA DE CARDIOLOGIA, (1996 Nov) 15 (11) 787-91, 771-2.

Journal code: AOW. ISSN: 0304-4750.

CY Portugal

DT Journal; Article; (JOURNAL ARTICLE)

LA English

EM 199704

EW 19970403

L10 ANSWER 21 OF 41 MEDLINE

AN 96403003 MEDLINE

DN 96403003

TI Prevention of restenosis after coronary angioplasty with low-density lipoprotein apheresis.

AU Adachi H; Niwa A; Shinoda T

CS Department of Medicine, Musashino Red Cross Hospital, Tokyo, Japan.

SO ARTIFICIAL ORGANS, (1995 Dec) 19 (12) 1243-7.

Journal code: 8ZK. ISSN: 0160-564X.

CY United States

DT (CLINICAL TRIAL)

(CONTROLLED CLINICAL TRIAL)

Journal; Article; (JOURNAL ARTICLE)

LA English

FS Priority Journals

EM 199702

EW 19970204

L10 ANSWER 11 OF 41 MEDLINE

AN 1998076997 MEDLINE

DN 98076997

TI Adenovirus gene therapy for \*\*\*hypercholesterolemia\*\*\*, thrombosis and \*\*\*restenosis\*\*\*

AU Gerard R D; Collen D

CS Center For Transgene Technology and Gene Therapy, Flanders Interuniversity Institute for Biotechnology, Katholieke Universiteit Leuven, Belgium.

SO CARDIOVASCULAR RESEARCH, (1997 Sep) 35 (3) 451-8. Ref: 69

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CY Netherlands

DT Journal; Article; (JOURNAL ARTICLE)

General Review; (REVIEW)

(REVIEW, TUTORIAL)

LA English

FS Priority Journals

EM 199803

L10 ANSWER 31 OF 41 MEDLINE

AN 95005582 MEDLINE

DN 95005582

TI Comparison of three porcine \*\*\*restenosis\*\*\* models: the relative importance of \*\*\*hypercholesterolemia\*\*\*, endothelial abrasion, and stenting.

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## Review

# Adenovirus gene therapy for hypercholesterolemia, thrombosis and restenosis

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## 1. General properties of recombinant adenovirus vectors

Human adenoviruses are non-enveloped, icosahedral viruses, approximately 130 nm in diameter. The linear, double-stranded DNA viral genome containing covalently attached terminal protein derived from a unique replication process is almost 36 Kb in length. Although forty nine serotypes of human adenovirus in six serological subgroups have been identified, only serotypes 2 and 5 of subgroup C have been extensively employed as gene transfer vectors.

Recombinant helper-independent adenovirus vectors are easily constructed, analyzed and propagated using standard recombinant DNA and virological techniques (reviewed in [1,2]). Foreign genes can be inserted into the adenovirus genome in a variety of locations to generate recombinant vectors, although substitution of early region 1 (E1) has been most widely used. Replacement of E1, since it is required for the efficient expression of the remainder of the viral genome, generates a vector which can only be propagated in a complementing cell line which supply the missing E1A functions in trans from an integrated copy of the appropriate fragment of the viral genome. Such E1 replacement vectors are typically propagated in the 293 [3] or 911 [4] cell lines. Recombinant viruses produced in this manner can be stably propagated to a titer of  $10^8$ – $10^9$  plaque forming units per ml and are readily purified free of

contaminating viral proteins and empty capsids using CsCl density gradient centrifugation for in vivo use [2].

Recombinant adenoviruses are highly efficient vectors for the transfer of foreign genes into cells both in vitro and in vivo. Because adenovirus vectors can infect a broad range of mammalian cell types, they have become the preferred vector for a wide variety of in vivo applications [5] and are currently approved for use in a limited number of human gene therapy trials. The high efficiency of adenoviral mediated gene transfer results from (i) the availability of purified, high titer adenovirus stocks that permit the transduction of the large number of cells within the tissues of an adult mammal, and (ii) the capacity of adenovirus to infect and express foreign genes in both actively dividing and quiescent cells.

E1 replacement adenovirus vectors have been historically used with strong viral promoters to drive transgene expression and thereby achieve significant levels of protein both in vitro and in vivo. However, the inclusion of cellular transcriptional regulatory sequences in the transgene permits regulated expression of the structural gene in a variety of studies. Both promoter strength and specificity are maintained when included in the foreign transcription unit and the gene can appropriately respond to various intracellular and extracellular signals [6–14]. In this way, targeting transgene expression to particular cell types can be accomplished, thereby limiting the potential for undesirable side effects due to inappropriate gene expression in non-target tissues.

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## 2. Adenovirus infection *in vivo*

### 2.1. Systemic gene transfer into liver

Following intravenous injection of recombinant adenovirus, the virus is preferentially cleared by the liver which leads to highly efficient gene transfer and expression in both hepatocytes and endothelial cells of that organ. Examination of the tissue distribution of foreign gene expression in mice following direct intravenous administration of a recombinant adenovirus encoding the firefly luciferase enzyme showed that more than 99% of all detectable luciferase activity was found in the liver, although all tissues examined contained low levels of activity [15]. Injection of an adenovirus vector expressing  $\beta$ -galactosidase demonstrates dose-dependent viral gene transfer into hepatocytes, with over 90% of liver cells in an adult mouse transduced with  $3\text{--}4 \times 10^9$  pfu of virus. This high efficiency of liver gene transfer is a result of the ability of the adenovirus particles to access virtually all hepatocytes via the fenestrae of the discontinuous endothelium in the liver sinusoids. These fundamental observations have been confirmed in a variety of other mammalian species, including rabbits [16], hamsters [17] and rats [18].

### 2.2. Local gene transfer into the vessel wall

From a cardiovascular perspective, the vessel wall is also an attractive target for gene therapy due to its direct involvement in clinically important pathologic syndromes of atherosclerosis, thrombosis and restenosis. Several studies have demonstrated that local infusion of viral vectors leads to localized gene transfer and expression in the vessel wall. The intraluminal dwell of adenoviral vectors following direct instillation into an isolated vessel segment leads to genetic modification of virtually all of the endothelial cells, but penetration of virus to deeper cell layers is usually not observed [19,20].

A catheter is also an efficient means of virus delivery to an isolated vessel segment, although leakage of virus solution into side branches and the circulation might lead to inappropriate gene expression in non-target tissues, particularly the liver. The various catheter designs currently available (double balloon, perforated balloon (Wolinsky), hydrogel-coated balloon, Dispatch<sup>TM</sup>, Infiltrator<sup>TM</sup>, etc.) can significantly influence the spatial pattern of gene expression. Normal arteries infected with adenovirus delivered via a double-balloon catheter show selective genetic modification of the vascular endothelium and of the vasa vasorum within the adventitia in a pattern similar to the intraluminal dwell [20–23]. In balloon injured arteries with an intact internal elastic lamina, the local infusion of virus can selectively target gene transfer to the developing neointima, particularly if gene transfer is delayed for several days following injury [24]. Introduction of virus into the deeper cell layers of the media and

adventitia of the vessel wall thus requires disruption of the physical barriers imposed by the continuous endothelium and the internal elastic lamina. This can be accomplished using high-pressure delivery of the virus solution with a perforated balloon or other catheter, although the frequencies of gene transfer and the distribution patterns vary widely for different catheter designs [20,25]. Viral access to deeper cell layers is primarily via fissures and dissection planes, although some diffusion will also occur. Various other factors can also influence the frequency of gene transfer, including virus purity, titer and volume, duration of exposure of cells to the virus solution, and inflation pressure required to maintain a seal of the device within the vessel can influence the degree of vascular trauma.

Balloon-injured and atherosclerotic arteries thus show a qualitatively different pattern of gene transfer from normal vessels, and this pattern is relatively independent of the type of delivery device [25,26]. Genetically modified vascular smooth muscle cells and foam cells are usually found in clusters adjacent to dissection planes within the neointima, media and adventitia where virus has gained access to cells within the vessel wall, even when a double-balloon catheter is used. Even small defects in the internal and external elastic laminae will allow penetration of adenovirus to deeper layers. However, catheter design can favourably influence the frequency of transduction as some designs deliver a greater volume of virus solution. These results demonstrate that the regions of the vessel wall in which gene transfer is observed are the same ones which are exposed to the circulation and which may contribute most to the cellular migration and proliferation responses observed during the stenotic process.

## 3. Adenovirus gene therapy of pathologic processes

~~The pathophysiological sequelae of atherosclerosis, thrombosis and stenosis are all potential targets for gene therapy using either systemic gene transfer to the liver or local gene transfer into the vessel wall. The problem is to identify appropriate therapeutic genes that can be efficiently delivered and expressed, and which will produce a biologically significant effect on those cellular processes contributing to the development of vascular disease [27].~~

### 3.1. Hypercholesterolemia

Elevated concentrations of low density lipoproteins (LDL) in blood are an important risk factor for the development of atherosclerotic vascular disease, whereas high concentrations of high density lipoprotein (HDL) are protective against this process. Therefore, numerous attempts to modify lipoprotein metabolism using gene transfer have been made (reviewed in [28,29]). A variety of strategies to reduce LDL cholesterol and/or elevate HDL and thereby reduce the risk of developing vascular disease have re-

cently been employed using intravenous adenovirus injection (reviewed in [30]). Adenovirus gene transfer has been targeted to the liver for two reasons. First, the large mass of this organ, its ability to be efficiently transduced by recombinant adenovirus and its central position within the circulation render it an attractive target for gene therapy to significantly alter circulating levels of lipoproteins. Second, the liver normally serves as the site of cholesterol regulation and transfer of a single gene involved in lipoprotein metabolism can effectively interact with the other key regulatory molecules involved in this process.

Numerous genes have been shown to transiently lower the lipoproteins LDL and VLDL in the circulation following adenoviral gene transfer. These genes are all involved in the transport, uptake and degradation of LDL-cholesterol by the liver. Increased LDL receptor expression will accelerate the rate of uptake of LDL/IDL particles by the liver and significantly reduce plasma cholesterol levels in Watanabe heritable hyperlipidemic (WHHL) rabbits [16,31] as well as in both normal and LDL receptor-deficient mice [15,32]. Gene transfer of the homologous VLDL receptor cDNA accelerates hepatic uptake of LDL/IDL particles, reduces plasma cholesterol by 50%, and corrects the abnormal lipoprotein profile in LDL receptor-deficient mice [33,34]. Following systemic gene transfer, the VLDL receptor can also clear the apoE2 and apoE3Leiden variant proteins and reduce plasma cholesterol by over 50% [35]. Gene transfer of apolipoprotein E (apoE) results in clearance of apoE-containing lipoprotein particles in apoE-deficient mice and normalization of the lipoprotein profile [36,37]. A reduction in the development of aortic atherosclerosis, which is accelerated in these animals, was also observed. Recently, the transfer of apoAI has been reported to have a similar effect on neointima formation following injury in this mouse strain [38].

Apolipoprotein B editing enzyme catalytic subunit (apoBEC-1) is responsible for the conversion of the mRNA encoding apoB100 to the form encoding apoB48. Reduction of plasma apoB100 and LDL is virtually complete following apoBEC-1 adenovirus-mediated liver gene transfer in normal mice [39]. In WHHL rabbits, apoBEC-1 gene transfer also affects VLDL particle size and modestly reduces total plasma cholesterol levels [40,41].

One of the most dramatic reductions in plasma cholesterol is accomplished via adenovirus-mediated gene transfer of the liver 7 $\alpha$ -hydroxylase cDNA in hamsters [17]. This occurs even though HMG CoA-reductase is induced, liver cholesterol synthesis is upregulated and the endogenous 7 $\alpha$ -hydroxylase gene is repressed. Animals fed both normal and high fat diets showed 60–75% reductions in total plasma cholesterol. Interestingly, the LDL receptor pathway was also upregulated, presumably to compensate for the increased rate of cholesterol elimination from the liver.

Hepatic lipase functions as an endothelial-bound phospholipase and triacylglycerol hydrolase and is necessary

for the proper metabolism of both IDL and HDL. In hepatic lipase-deficient mice, adenoviral gene transfer of the hepatic lipase cDNA results in 50–80% decreases in total plasma cholesterol, triglycerides and phospholipids, as well as a normalization of the lipoprotein profile in these animals [42].

Genes shown to raise the levels of anti-atherogenic proteins have also been transferred *in vivo* using adenovirus vectors (reviewed in [30,43]). The apolipoprotein A1 (apoAI) cDNA was shown to be highly effective in elevating plasma levels of apoAI which could be assembled into HDL particles in hamsters [44], and in mice [37]. This results in a marked increase in circulating HDL. A significant augmentation in the levels of apoAI and HDL particles via an indirect mechanism following gene transfer of the LDL receptor in WHHL rabbits has also been observed [31,40]. Systemic overexpression of human lecithin-cholesterol acyltransferase (LCAT) in mice transgenic for human apoAI results in elevated LCAT levels (200-fold increase), plasma HDL cholesterol levels (6-fold) and apoAI levels (2-fold) [45]. The size and surface charges of the HDL particles in these mice was also modified by LCAT overexpression. Most importantly, this study directly demonstrated that serum from LCAT virus-injected animals can increase cholesterol efflux from cells *in vitro*, an effect that was dependent on the level of plasma apoAI in the medium, thus providing important evidence that LCAT and apoAI can mediate reverse cholesterol transport from peripheral tissues.

These results in animal models demonstrate the utility of adenovirus vectors in altering plasma lipoprotein profiles to favourable influence the progression of atherosclerotic disease. Whether single genes or combinations of genes will ultimately provide the most effective form of therapy, and whether systemic intravenous infusion into humans can be safely and effectively performed remains at issue.

### 3.2. Thrombosis

Currently available antithrombotic therapy includes the use of systemically administered anticoagulant and/or thrombolytic agents. For some life-threatening acute thrombotic syndromes, e.g. acute myocardial infarction, stroke or pulmonary embolism, short-term administration of a thrombolytic agent combined with anticoagulation is an effective therapeutic regimen. However, prolonged administration of thrombolytic agents for subacute episodes of thrombosis during sepsis, trauma or surgery is impractical. The transient overexpression of genes whose products are directly involved in the coagulation cascade or fibrinolytic system might achieve a more potent and sustained antithrombotic state.

Conceivably, gene transfer to treat vascular thrombosis could be directed either locally or systemically. In a vessel at risk for the development of an occlusive thrombus, for

example, in syndromes of coronary artery disease and acute myocardial ischemia, direct genetic modification of cells in the target vessel wall may be preferable. This would both limit the potential for deleterious systemic effects and direct the expression of the therapeutic protein to the site where it is most needed. In the setting of a systemic hypercoagulable state, however, effective prophylaxis or therapy might benefit from a systemic approach. Both alternatives can be achieved with local or systemic gene transfer of adenovirus vectors.

For example, genes regulating the coagulation cascade might be used as a prophylactic measure to inhibit the formation of intravascular blood clots. Theoretically, a wide array of gene products could be applied therapeutically to interfere directly with the coagulation cascade or to alter platelet aggregation. Recently, the adenovirus-mediated gene transfer of the human cyclooxygenase 1 cDNA to injured carotid arteries was found to be a highly effective local treatment that prevented cyclic flow changes and thrombus formation by raising prostaglandins, inhibiting platelet aggregation and maintaining vascular SMC relaxation [46]. Similarly, the overexpression of thrombomodulin on endothelial cells in vitro via gene transfer with recombinant adenovirus enhances the activity of activated protein C and inhibits clot formation on activated endothelial surfaces [47]. This may ultimately prove to be a useful strategy to inhibit clotting in vivo by feedback inhibition.

Alternatively, genes directly involved in the fibrinolytic cascade might be used to dissolve the fibrin meshwork of a blood clot that has already formed. For example, gene transfer of tissue plasminogen activator (t-PA) might be expected to have a beneficial effect on thrombolysis. One test system used both mice homozygous for the targeted disruption of the endogenous t-PA gene [48] and transgenic mice overexpressing PAI-1. Both animal models demonstrated defective lysis of  $^{125}$ I-labelled fibrin clots in a pulmonary embolism model. Adenovirus-mediated transfer was performed into the liver using both wild type t-PA, as well as a serpin-resistant human t-PA, which demonstrates clot lysis activity substantially greater than that of the native enzyme in the presence of physiologic concentrations of PAI-1 [49]. Circulating t-PA antigen and activity levels were augmented in a virus dose-dependent manner, and both the wild type and variant forms of the enzyme increased the extent of clot lysis. These results suggest that transient overexpression of t-PA, which occurs as early as four hours following gene transfer and can persist for over one week in animal models, may eventually prove an effective strategy for the treatment of subacute thrombotic syndromes.

### 3.3. Restenosis

Genes affecting local cellular proliferation, migration and neointimal formation in the vessel wall might favourably affect the outcome of the stenotic process. In

principal, gene products affecting vascular tone may also influence the sequelae of injury to the vessel wall.

A variety of different genes encoding antiproliferative proteins have already been delivered to cells within the vessel wall and shown to reduce neointima formation. They include proteins that act directly on smooth muscle cell proliferation such as cell cycle proteins, toxic gene products, or products of normal endothelium. Alternatively, indirectly-acting gene products like vascular endothelial growth factor have been used. The cellular targets of gene transfer have been both endothelium and smooth muscle cells within the media and adventitia.

The retinoblastoma (*Rb*) gene product functions as a cell cycle inhibitor to inhibit the proliferation of smooth muscle cells that characterize the proliferative response. Adenovirus-mediated gene transfer of a constitutively active, non-phosphorylatable form of *Rb* has been shown to reduce smooth muscle cell proliferation and restenosis in two animal models following balloon angioplasty [50].

The p21 protein is an important negative regulator of cell cycle progression in mammalian cells that functions by inhibiting cell cycle dependent protein kinases and by binding to proliferating cell nuclear antigen, a DNA polymerase  $\delta$  cofactor. Adenovirus encoding the p21 protein has been used to locally infect balloon-injured rat carotid arteries and reduce vascular smooth muscle cell proliferation and neointima formation [51].

Similarly, *gax* is a homeobox gene normally expressed by vascular smooth muscle cells that is downregulated following vascular injury to promote the wound-healing response in the vessel wall. In balloon-injured rabbit iliac arteries, adenovirus-mediated *gax* overexpression in smooth muscle cells prevents neointimal hyperplasia and luminal stenosis, but does not affect re-endothelialization and endothelium-dependent vasomotion [52].

The herpes simplex virus thymidine kinase (tk) gene functions as a selectively toxic gene product when combined with ganciclovir treatment and kills transduced cells, thereby depleting the population of proliferating cells within the target tissue. Local adenovirus-mediated gene transfer of tk has been demonstrated to reduce neointima formation by 50% in injured porcine arteries [53], and the BrdU labelling index by 65% and neointima formation by 50% in a rabbit hyperlipidemic injury model [54].

Vascular endothelial growth factor (vegf) is an endothelial mitogen which acts to promote re-endothelialization of the vessel wall, limit the exposure of underlying smooth muscle cells to mitogenic stimuli, and thereby reduce the neointimal proliferation of smooth muscle cells following balloon injury of normal vessels. In a rabbit model of atherosclerosis, local adenovirus-mediated vegf gene transfer has been shown to increase the minimal luminal diameter and thus reduce the degree of post-angioplasty stenosis [55]. In another study, an adenovirus vector expressing the specific clotting inhibitor hirudin was used to locally transduce smooth muscle cells and inhibit active thrombin in an

injured rat carotid model [56]. Systemic effects on clotting were not observed as evidenced by unchanged partial thromboplastin times, but neointima formation in the vessel was inhibited by 35%. The rationale underlying this study is that the inhibition of thrombin reduces the exposure of the injured vessel segment to cytokines and/or growth factors released locally at the site of injury by activated monocytes, endothelial cells, or platelets within a blood clot.

The constitutive endothelial nitric oxide synthase (ceNOS) enzyme is responsible for the local production of nitric oxide (NO). ceNOS gene transfer to smooth muscle cells in rat carotid arteries denuded of endothelium by balloon injury can also have profound local effects. By increasing cGMP levels and inhibiting smooth muscle cell proliferation at the site of injury, ceNOS gene transfer is an effective therapy to reduce neointima formation within the injured segment [57].

Theoretically, antimigratory proteins can also be used to inhibit the process of smooth muscle cell migration that characterizes cellular remodelling of the vessel wall. The serpin plasminogen activator inhibitor 1 (PAI-1), a specific inhibitor of both urinary plasminogen activator (uPA) and tPA, is an attractive candidate protein to treat vascular stenosis based on the results of gene targeting experiments in mice. The results obtained with vascular injury models in mice lacking genes encoding various components of the fibrinolytic system demonstrate that plasminogen activation is involved in the process of smooth muscle cell migration and vascular stenosis ([58]. Further, mice lacking urinary plasminogen activator (uPA) responsible for cellular plasminogen activation and degradation of extracellular matrix show significant reductions in the vascular wound healing response that leads to stenosis, while conversely, plasminogen activator inhibitor-1 (PAI-1) deficient mice are hyperstenotic and show accelerated smooth muscle cell migration and proliferation responses. PAI-1 gene transfer into PAI-1 knockout mice has been performed using adenovirus-mediated gene transfer into the liver to augment systemic expression of PAI-1 [59]. The results demonstrate that restoration of circulating PAI-1 into such mice can suppress the vascular wound healing response and dramatically reduce the degree of vascular stenosis which develops following injury. Interestingly, no deleterious side effects on homeostasis by PAI-1 overexpression have been observed in these mice.

In related studies on degradation of the extracellular matrix by vascular smooth muscle cell expression of metalloproteinases, adenovirus-mediated overexpression of the tissue inhibitor of metalloproteinase-2 (TIMP-2) was found to inhibit smooth muscle cell migration through Matrigel in vitro [60], which may also prove to be a useful strategy in vivo. Since plasminogen activation has been shown to activate matrix metalloproteinases, this observation supports the hypothesis that uPA is a primary mediator of smooth muscle cell migration and neointimal thickening.

Conversely, gene transfer in normal rabbits has been shown to increase neointimal thickening and augment contractile responses in vein grafts transduced by a recombinant adenovirus vector expressing TGF $\beta$ 1 [61]. These effects can be explained by an increase in local plasminogen activation in the TGF $\beta$ 1-transduced vessels which increases smooth muscle cell migration and proliferation in the vessel wall.

Genes whose expression will alter vascular tone can also have significant effects on blood vessel remodelling and other processes. For example, NO or endothelium-derived relaxing factor, has potent relaxation properties on blood vessels. Instillation of adenovirus into the lumen in normal rabbit vessels and endothelial cell gene transfer using virus encoding ceNOS results in augmented ceNOS synthesis, increases in basal cGMP levels, and both diminished contractile responses to phenylephrine and enhanced relaxations to acetylcholine [62]. Similarly, gene transfer of  $\beta$ 2-adrenergic receptors was shown to augment expression six-fold and thus enhance the vasorelaxation induced by isoproterenol in de-endothelialized rat carotid arteries [63]. Conversely, adenoviral gene transfer of the endothelin cDNA to the livers of rats led to pathophysiologic levels of endothelin expression and systemic hypertension mediated by the ET $_A$  receptor [64].

Despite these positive results, other groups have claimed that adenoviral gene transfer *per se* into rabbit arteries can produce cellular activation, inflammation and neointimal hyperplasia [65]. These processes are induced by viral infection and are independent of the expression of a foreign protein from the transduced gene. Since these effects have not been observed in other species of experimental animals, it is not clear that these responses are general phenomena. Whether the reduction in proliferation observed in rabbits or other mammalian species using therapeutic genes is offsetting that induced by exposure to the virus also remains to be determined.

#### 4. A perspective on the use of adenovirus vectors for gene therapy

Combined with transgenic mice in which genes have either been overexpressed or deleted, recombinant adenovirus vectors have both provided a powerful investigatory tool and allowed new insight into the molecular mechanisms of atherosclerosis, thrombosis and stenosis. This technology also provides an opportunity to develop therapeutic modalities for these disorders and test them for efficacy in relevant animal models of human disease prior to clinical trials. In many cases, the positive results obtained in animal experiments with pharmacologic treatment regimens have not proven to be particularly effective in human subjects, probably because the animal models only approximate the complexity of the human disease process. Nevertheless, the results of gene transfer obtained

with relevant animal models may favourably extrapolate to human subjects and thereby catalyze the development of effective forms of therapy for hypercholesterolemia, thrombosis and restenosis.

While recombinant adenoviruses have a number of characteristics that render them attractive as potential therapeutic agents, gene transfer and expression from first-generation vectors is of brief duration, which limits their therapeutic efficacy. There appear to be three significant problems to be overcome before human gene therapy with these vectors will develop into an effective modality. First, immediate inflammatory reactions to the infecting viral capsids in the transduced tissue seem to both prime the immune system and augment secondary immune responses to viral gene therapy. Immune reactions can either limit or augment the expression levels during the first few days if appropriately responsive promoters are used to direct expression [12]. If hepatic gene therapy in humans is to be realized, it will be necessary to minimize such inflammatory reactions. Second, humoral neutralizing antibody responses to the viral capsid antigens effectively limits systemic adenovirus gene transfer to a single therapeutic dose, and gene delivery via other routes of administration can also be significantly reduced. Antibodies to the foreign protein expressed by the vector sequences can also result in the efficient neutralization and clearance of the desired protein product from the circulation or the cell surface [66]. Immune responses to intracellular protein products such as  $\beta$ -galactosidase have also been reported [67,68]. Third, cellular immune responses can result in the loss of transduced cells. Coexpression at low levels in transduced hepatocytes of viral proteins leads to their CTL-mediated destruction and repopulation of the tissue by non-transduced cells [69]. CTL responses to the expressed transgene can also be observed [67]. It is thus apparent that the host immune response to recombinant adenovirus transduced cells can severely limit both the efficiency and the duration of therapeutic expression from recombinant adenovirus vectors.

For investigational and some therapeutic purposes, the transient expression of foreign genes may be sufficient. Whether gene expression from such vectors can ultimately persist for sufficient time to exert the desired physiological effect is a challenge to be met by additional experimentation. Recombinant adenovirus vectors have solved the problem of efficiency of gene transfer which is of utmost importance in gene therapy. For many applications, the development of strategies to achieve stable, long-term expression of foreign genes is a key factor in the application of recombinant adenovirus to human gene therapy.

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